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Concerted Action "Improvements of Tagging Methods for Stock Assessment and Research in Fisheries" (CATAG)

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7. FISH WELFARE AND HEALTH IN RELATION TO TAGGING

7.1. INTRODUCTION

All forms of fish tagging involve invasive procedures, first by capture itself. Externally-fixed, or superficially-injected tags breach the skin and musculature, while internal tags (whether mounted in the stomach or peritoneal cavity) normally involve either force feeding or surgery (though some tags can be ingested voluntarily in food/bait). Use of anaesthesia may itself alter body biochemistry (e.g. MS222 use causes elevated serum cortisol levels in coho salmon; Strange & Schreck, 1978). All types of tags have the potential to cause health problems for fish subsequent to the tagging process itself. There may be disturbances of physiological function, or more subtle behavioural or immunological effects.

7.2 ANAESTHESIA

7.2.1. Introduction

Rendering fish quiet (sedation) or unconscious (anaesthesia) is crucial to several aspects of fish tagging. Summary sheets at the end of this section are intended to help operators choose and use anaesthetics: they are also readily downloadable as OHP slides. More information about anaesthesia may also be gained by interrogating the WELFARE database. Operators should be aware that there are legislative implications of use of anaesthetics on fish that are to be released to the wild because

of the perceived risk of chemical residues reaching humans through the food chain (see section 6.2.4 in legislation section).

7.2.2. Anaesthesia

A variety of handling methods have been applied during the tagging process, ranging from use of blindfolding in calming fish, to full anaesthesia involving continuous irrigation of the gills with fresh or seawater containing diluted anaesthetic agents.

Under anaesthesia, handling stress will be reduced and tagging can be accomplished more rapidly without risk of the fish hurting themselves when trying to escape. Although the use of anaesthetics in some cases may be unwanted due to their detrimental effects on the physiology and behaviour of the fish, considerations of animal welfare will in most cases prohibit tag attachment to unsedated fish if surgery is involved.

7.2.3. Choice of anaesthetics

Different handling procedures demand different anaesthetic approaches. Light anaesthesia (=sedation) is defined as 'reduced activity and reactions to external stimuli', and is sufficient for procedures such as transport or weighing of fish. Full anaesthesia can be defined as 'loss of consciousness and reduced sensing of pain, loss of muscular tonus and reflexes' and is needed when surgical procedures are applied (MacFarland 1959).

The behavioural changes occurring in fish passing through sedation to full anaesthesia were classified by MacFarland (1959). There are 4 stages with subclasses ranging from normal (stage 0), where the fish reacts to external stimuli and where the muscular tonus and swimming ability is normal, to the stage of total physiological collapse (stage IV), where gill movements have stopped and which in a few minutes will lead to heart failure. In a tagging context, the stages where the fish is in a state of light/deep anaesthesia (stages II and III) are of greatest relevance, as the animal is then insensitive to pain caused by the attachment of transmitters or data storage tags.

Choice of sedatives/anaesthetics must be based on the species to be tagged, the number and size of fish involved, and the duration of the operation in question. Water temperature and chemistry have also to be taken into consideration when choosing the method. Lastly, the work often has to be done under primitive field conditions without accurate control of concentrations and exposure times. An anaesthetic with a good safety margin between effective anaesthesia and irrevocable collapse is essential in such circumstances.

7.2.4. Categories of methods

(a) Physical sedation methods

Physical sedation can be obtained by rapid lowering of temperature or by electric shock. The former method is mainly applicable for transportation (c.f. Ho & Vanstone, 1961). Coldwater adapted species, and marine fish require lower temperatures for sedation than warm water species and freshwater fish (Chung 1980). Water cooling can also be used in conjunction with other anaesthetics (e.g. Benzocaine) but the dosage must then be reduced by about 30% (cf. Ross and Ross 1983). Electroanaesthesia has a number of advantages such as rapid immobilisation of fish, no need for chemicals, rapid regain of consciousness and low costs (Madden and Houston 1976, Gunstrom and

Bethers 1985, Tytler and Hawkins 1981; Cowx & Lamarque, 1990; Cowx, 1990). But these are outweighed by the fact that the method cannot be used in saline water, and the danger of using inappropriate voltage levels, which may give severe physiological stress responses in experimental fish (Shreck *et al.* 1976) due to hypoxia. There are also significant risks to experimenters, principally from electric shock. In the U.K. the National Rivers (NRA) issued a safety Code of Practice in 1995.

(b) Chemical sedation and anaesthesia

Chemical sedation is distributed to fish in liquid dilutions of varying strengths depending on the agent used. The sedative is inhaled by the fish and diffuses across the gill epithelia. In minor quantities it can also diffuse into the fish via the skin (Ferreira *et al.* 1984) - this may be a particularly significant route in scaleless fish with well-vascularised skins. Since these chemicals are absorbed and excreted predominantly via the gills, fish with a large surface of gill epithelium for a given body weight (e.g. salmonids) require lower doses of anaesthetics than fish (e.g. eels) with relatively smaller epithelial surfaces (Ross & Ross 1983). Other factors affecting the absorption and excretion of chemicals are the relationship between the surface of the gill epithelium and the body volume, thickness of epithelium, type of anaesthetic, dosage and temperature.

All known anaesthetics have unwanted side effects. Most of them are barbiturates, which lead to unconsciousness, inhibition of the sensing of pain and loss of muscular tonus and reflexes. The most important complication connected with all forms of chemical anaesthesia is hypoxia due to reduced respiration and vascular activity. This leads to physiological changes in the blood (e.g. lowered pH), hypotonia (= reduced blood pressure), raised blood glucose, blood lactate and haematocrit (Tytler & Hawkins 1981). In addition to physiological deterioration of blood parameters, hypoxia can cause brain damage, which interferes with directional orientation (Taylor 1988), or alters temperature preferences (Goddard *et al.* 1974).

Widely used anaesthetics of the barbiturate group are:

MS 222- Tricaine methane sulphonate

Chemical name: ethyl- amino- benzoatemethanesulphonate. MS 222 is probably the most widely used fish anaesthetic world-wide, and there are numerous studies on the physiological effects of this agent (e.g. review by Bell 1987). It is a crystalline powder easily dissolved in fresh and seawater. The recommended dosage for anaesthesia is 50- 100 mg/l (Klonz 1964; Ferreira *et al.* 1979). It should be observed that MS222 becomes toxic in seawater exposed to sun (Bell 1987). MS222 gives an acid solution and a dosage of 75 mg l⁻¹ can cause the pH to fall to 4.0 in soft water (Wedemeyer 1970). This effect can, however, be mediated by adding 5- 6 ml saturated (10%) solution of NaHCO₃ to 1 litre of 100 mg l⁻¹ solution of MS222.

Benzocaine

Chemical name: Ethyl-p-aminobenzoate. This chemical is also very widely used in fish anaesthesia. It is chemically close to MS- 222, both being derivatives of p- aminobenzoic acid. Benzocaine is a white crystalline powder, which is insoluble in water and has to be dissolved in ethanol in a 'master solution' of 1 g l⁻¹ 96% alcohol. The master solution should be stored in a dark bottle, and has a life of up to a year. The recommended dosage is 2.5 ml of this master solution to 10 l of aerated water. With this dosage the animals should be immobilised in 2 - 5 min. and the recovery time will be 5 - 15 min. Benzocaine gives a neutral solution (Egidius 1973). The time to obtain anaesthesia was

observed to take 1.5 min longer time for trout (*Salmo trutta*) and 3 min longer for pike (*Esox lucius*) in 7° C water than at 12 ° C (Dawson & Gilderhus 1979). According to Wedemeyer (1970) a comparison between Benzocaine and MS-222 as anaesthetics for salmonids was slightly in favour of Benzocaine as less metabolic change was observed. More recent studies by Soivio et al. (1977) showed few differences between the two; both caused hyperglycaemia. However, benzocaine caused somewhat lesser hyperglycaemia than MS- 222. With the exception of occasional allergic reactions, health hazards to humans are not normally recorded with the use of benzocaine (MND 1986).

Chlorbutanol- Chlorbutol- Chorethone- Acetochloroform

Chemical name: Chlorbutanol. Although classified as a safe anaesthetic for fish (Johansson 1978), it has not been widely used outside Scandinavia due to health hazards to humans connected with its use. Inhalation of larger quantities may cause unconsciousness, it can also irritate human skin and eyes. Chlorbutanol (Cb) is a crystalline colourless powder that has to be dissolved in ethanol. The usual base solution is 30 g to 100 ml 96% ethanol, and the dose 10 ml base- solution to 10 litres aerated water. Johansson (1978) states that the time for falling into stupor and wakening is inversely dependent to the water temperature, the higher the temperature the lesser the time needed for sedation. The dosage varies somewhat with the size and species of fish but is considered sufficient when the fish rolls on it side after 3-5 min. Chlorbutanol gives a light anaesthesia, but it is normally sufficient when the fish only needs to be handled for a short time handling, such as in tagging (Johansson 1978, Horsberg and Høy 1989). Chlorbutanol is considered a safe anaesthetic for fish, although a study by Hansen and Jonsson 1988 showed an 87 % reduction in return rates of Atlantic salmon (*Salmo salar*) smolts anaesthetised before release in comparison with untreated fish. Chlorbutanol has also been tested on Atlantic halibut (*Hippoglossus hippoglossus*), but with a dosage of 50 ml base solution dissolved in 10 l water. The smallest fish are most rapidly sedated; they also have the shortest recovery time.

Methomidate chloride

Methomidate is a hypnotic (sleeping-agent) and not a barbiturate. It therefore causes less depression of respiration than MS-222 or Benzocaine. This may lead to fewer and less serious side-effects. Methomidate is water-soluble. Mattson & Riple (1989) report an effective concentration of 5 mg l⁻¹. Methomidate was tested on rainbow trout in the early 1980s by Gilderhus & Marking (1987), and showed in these tests to give a relatively long wake-up time and also some mortality after treatment. However, during the late 1980s this anaesthetic has been tested with good results for handling salmonids and other fish in culture, such as cod and halibut at the Department of Aquaculture, Institute of Marine Research, Norway, (Mattson & Riple 1989; Huse, pers. Com.; Furevik, pers. com). From 1992 onwards methomidate has been the only anaesthetic used at the Dept. of Aquaculture (Holme, pers. com.); the only negative feature is the high cost of the product.

Quinaldine

Quinaldine is not easily soluble in water, and is also reported to be irritating to human skin and mucus membranes. Quinaldine-sulphate does not have these negative effects, but gives an acid solution, and must therefore be buffered with sodium bicarbonate (Blasiola 1977). It has been used in acetone solution for the capture of intertidal fish living in rock pools. Reports that it may be carcinogenic currently restrict use.

Propanidide

In a 5% solution this chemical is water-soluble. *Propanidide* seems to have few physiological side effects, and can be used both for short- and long-duration anaesthesia. The main reported asset of this anaesthetic is that it does not reduce the ventilatory rate of the fish (Ross & Ross 1987). The blood-circulation can also remain unaffected as reported by Veenstra et al. (1987) from studies of *S. fontinalis* embryos and 7 days old alevins of amargosa pupfish (*Cyprinodon nevadensis amargosae*). It has also been tested on carp (Jeney *et al.* 1986) rainbow trout and smolts of Atlantic salmon and sea trout (Siwicki 1984) with good results.

Clove oil

Chemical name: eugenol (4-allyl-2-methoxy-phenol). Recent experiments (Anderson et al. 1997) have shown that clove oil is just as effective an anaesthetic for both juvenile and adult rainbow trout (*Onchorhynchus mykiss*) as MS-222. Clove oil does not affect swimming performance and it also provides swift induction and recovery from anaesthesia. It is regarded as a GRAS ('generally recognised as safe') substance by the US Federal Drugs Administration (FDA) and is suitable for use in field studies where immediate release of the fish into the food chain is required. Anderson *et al.* (1997) have shown that concentrations of 20-40 and 100-120 mg/l will induce light and heavy anaesthesia, respectively. At a concentration of 120 mg/l induction times are significantly faster than MS-222 for both juveniles and adults. At a concentration of 40 mg/l there is no difference for juveniles but induction times are significantly faster for adults. Recovery times for adult fish are rather longer than MS-222 at the higher concentration but no different at the lower concentration.

7.2.5. Information sheets (http://www.hafro.is/catag/f-health&welfare/studies-res_2.htm)

Downloadable information sheets that will assist in the choice of anaesthetics for specific purposes have been prepared; they are displayed in Appendix II (7.10) of this chapter and are also available on the CATAG web site (<http://www.hafro.is/catag>).

7.3. EFFECTS OF CONVENTIONAL TAGS ON FISH

Consideration of conventional tagging (including procedures such as fin-clipping) will be given here. Generally such tagging procedures are innocuous and there is little or no stress to fish beyond that involved in capture and handling (e.g. chinook salmon, *Onchorhynchus tshawytscha*, Sharpe *et al.*, 1998; see also Gjerde & Reftstie, 1988, Hansen, 1988). The main problem associated with tags is that of pathological lesions caused by tagging or fin clipping (Roberts *et al.*, 1973a, b, c; Morgan & Roberts, 1976), or indeed any breach of fish skin. Such lesions may be subject to secondary infections and are likely to cause effects on growth rate and reproductive performance. Uncontrolled infections may well be a source of mortality, but it seems probable that this is very rare.

Adipose fin clipping (commonly performed on Pacific salmon) may be detrimental because there is some evidence that these fins are secondary sexual characters, which perform an important function in mate selection.

Most tagging experiments are based on the assumption that the behaviour, growth and survival of tagged fish is similar to that in untagged fish and that data generated from these studies is unaffected

by the type of tag used or the tagging procedure implemented. Few studies have been carried out to assess the impact of simple external tags on the behaviour of fish (e.g. Lewis & Muntz, 1984; McFarlane & Beamish, 1990), probably because they are difficult to design and carry out. Furthermore, tag effects are sometimes examined under controlled laboratory experiments, which often provide conditions different from the natural environment.

While many of the internal tags or marks may have minimal or negligible effect on the behaviour of marked fishes (Buckley & Blankenship, 1990), external tags may affect the behaviour of tagged fish. Small individuals may have problems with relatively large tags and the application of the tag may cause problems, such as wounds around the attachment. External tags may affect feeding or evasive behaviour and the fish may therefore be more vulnerable to predation. Especially in demersal fish, tags may become overgrown with algae and/or mussels, becoming heavier and more cumbersome. An external tag that has not been anchored firmly into the muscle may continue to irritate the fish, preventing the wound from healing causing a chronic wound.

Growth of sablefish, *Anoplopoma fimbria*, was found to be affected by the tag or tagging procedure in a comparison of wild, tagged fish with untagged fish (McFarlane & Beamish, 1990). Thus, extrapolating growth information from tagged fish resulted in altered estimates for mortality and mean age at maturity for this species. On the other hand, no effect on growth was observed in similar studies with Arctic char (*Salvelinus alpinus*) (Berg & Berg 1990).

Carlin tagging and fin clippings are commonly used in studies on salmon or trout migration, survival or growth. Saunders & Allen (1967) showed negative effects of this tagging method on survival of Atlantic salmon, *Salmo salar*, implying that mortality estimated from tagged salmon smolts would result in an underestimation of the survival rates to adults. This was confirmed in later studies on the same species by Isaksson & Bergman (1978) and Hansen (1988). The increased mortality was attributed to handling, anaesthesia and marking of fish. Carlin tagging was found to have a higher impact on survival than fin clipping, although the latter was not without impact, probably due to stress from handling and anaesthesia. In a laboratory study on snapper (*Pagrus auratus*), no effect of dart tags on survival or growth was observed on three length sizes of fish during a one-year period (Quartararo & Kearney 1996).

All tagging or marking of fish involves treatment, which disturbs the fish and may stress or harm the fish. Careful handling procedures throughout the capture and marking process are of highest importance. Physiological research has shown fish to be stressed for a prolonged period after handling; for example, levels of lactic acid may be elevated for more than 24 hours after stressing the fish at certain temperatures (Wendt 1965, 1967; Wendt & Saunders, 1973). Histopathological studies on the effects of Disc-dangler tags on Atlantic salmon (Morgan & Roberts, 1976) revealed that external tags of these types can leave severe traumatic wounds which may lead to secondary infection. Similar observations were made by Vogelbein & Overstreet (1987), who reported histopathological problems with internal anchor tags used on spot, *Leiostomus xanthurus*. The incomplete healing of the integument during the life of the fish may affect the normal behaviour of the fish and result in biased estimates of biological parameters.

A possible (and virtually unstudied) effect of all types of external tagging (whether conventional or with electronic tags) is that tags may become fouled, causing enhanced drag, so disadvantaging the fish. Anecdotal evidence has been collected during CATAG of the existence of such fouling (e.g. by barnacles and seaweed) but more investigation is needed. In particular, it would be desirable if

systematic fouling trials could be conducted on tags and tag materials - it is quite possible that fouling could be a source of unremarked mortality of tagged fish.

7.4. EFFECTS OF ELECTRONIC TAGS ON FISH

7.4.1. Introduction

Electronic tags have become commonly used during the last decade to monitor movements, activity, physiological responses and reaction to a number of environmental variables in many fish species. The area of electronic tags is in rapid development, and these tags are used by an increasing number of teams and researchers, in an increasing number of species, most of which have never been tagged before. Implicit in these studies is the usual assumption that the tag and the tagging procedures have no significant effect on the data collected. Whereas some authors found no difference between tagged and untagged fish in terms of behaviour, growth or physiology (e.g. Hinch *et al.*, 1996), other studies have documented adverse effects that are dealt with here. Furthermore, only a very small proportion of tagging studies have investigated the actual adverse effects of tagging, and effects on behaviour or physiology have been investigated far less frequently than direct, 'obvious' effects on survival, anatomy or pathology (see Figure 7.1).

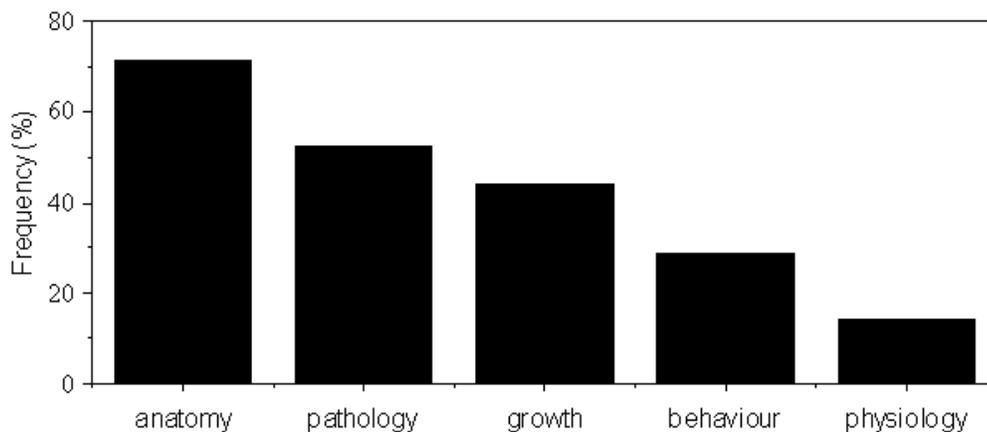


Figure 7.1. Proportion of tagging feasibility studies where the effects of tags or tagging procedure on anatomy, pathology, growth, behaviour and physiology were investigated.

A future goal should be to ensure that the effect of the tag and the tagging procedures on the animals used in any project are studied before this type of assumption can be made with confidence. Furthermore, because electronic tags and tagging techniques are developing rapidly, the need to document modifications of behaviour from newly developed techniques needs to be emphasised. This should be done, not only to secure the welfare of the animals, but also to avoid biased data collection due to decreased performance, altered behaviour or elevated stress level in the fish.

The present review focuses on the effects on fish of tagging and carrying electronic tags. Because of their larger size and mass, telemetry (radio and acoustic) and data storage tags (DST or archival tags) are considered separately from other electronic tags, such as passive integrated transponder (PIT) tags, and from conventional tags (see Section 7.3). The main results from studies dealing with the effects of radio and ultrasonic transmitters in fish are summarised in Appendix I (7.9) of this chapter. Additional, more detailed information can be found in the WELFARE database on the CATAG web site (<http://www.hafro.is/catag>).

7.4.2. Survival

For ethical considerations, cost effective research and reliable statistical analyses, it is crucial that fish survive the tagging procedure and that neither the tag nor the tagging procedure influence the survival rate of the fish, either during the time of the study or later. Survival rates evaluated in telemetry or DST tagging studies ranged from 20 % one month after tagging (grass carp *Ctenopharyngodon idella*, Schramm & Black, 1984) to 100 % 30 months after tagging (blue tilapia *Oreochromis aureus*, Thoreau & Baras, 1997). Because of differences between the procedures used by different authors (e.g. threads for attachment, coating, tag size, anaesthetics, temperature) and because not all factors likely to influence mortality are systematically investigated, or mentioned in feasibility or field studies, it may be difficult to draw general trends. Different fish species or life history stages may also have different resistances to handling or pathological outbreaks. However, the analysis of the CATAG fish WELFARE data base provides evidence that gastrically-inserted transmitters are less prone to cause the death of fish, compared with externally- attached or intraperitoneally-inserted transmitters (Figure 7.2). Surgical procedures are often deemed to be the most invasive ones, since they require deep anaesthesia, longer handling, opening of the body cavity, and insertion of a foreign body inside the fish. But carefully evaluated procedures tailored to the species of interest are frequently reported to cause no additional mortality compared with controls.

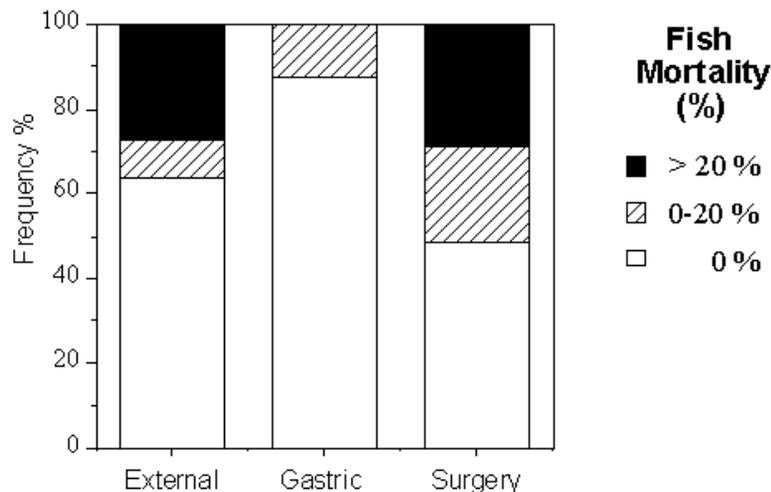


Figure 7.2. Proportion of telemetry studies reporting variable rates (0 %, < 20 %, 20 %) of fish mortality depending on attachment procedure.

Mortality of internally-tagged fish takes place most frequently within the hours, days or weeks following tagging, as a result of wound infection, blockage of gut transit or damage to internal

organs. Wood *et al.* (1983) reported that 40 % of tagged rainbow trout *Onchorhynchus mykiss* died within 12 hours following 6 min of intensive exercise, probably because of acidosis. Similarly, most cases of mortality of surgically-implanted fish took place before the fish had healed their incisions and recovered physical integrity and osmotic balance (within 4 days to 7 weeks, depending on species and ambient temperature). In contrast, deaths of externally-tagged fish rarely take place within the first days or weeks. External tag attachment involves progressive, or chronic lesions to muscular tissues, in which degenerative processes exceed by far the capacity for tissue repair (Roberts *et al.*, 1973; Brittles, 1995; Knights & Laze, 1996). Adverse effects thus accumulate over time and can be exacerbated by exposure to increased water velocity, which increases the drag on the tag. These problems can, however, be postponed depending on the time interval between the moment of tagging and the time of the year when the fish moves into a faster flowing environment. Externally-attached tags or trailing antennas may become entangled in vegetation (e.g. Chinook salmon, *Oncorhynchus tshawytscha* Adams *et al.*, 1998). This can cause tag shedding or fish mortality.

7.4.3. Retention

Tag shedding or expulsion has been reported for all three major attachment procedures (externally-attached, intragastrically-inserted, intraperitoneally-inserted), as well as for oviduct insertion, which has recently been evaluated in salmonids (Peake *et al.*, 1997). Generally, shedding has been reported more frequently, and shedding rates found to be higher for gastrically-inserted tags than for external or intraperitoneal tags (Figure 7.3), and this contrasts with the mortality rates inherent in these three procedures. This section will concentrate on shedding or expulsion mechanisms, and conditions that increase the propensity of fish to shed tags. Details on tag shedding rates in different species or life stages can be found in the WELFARE data base.

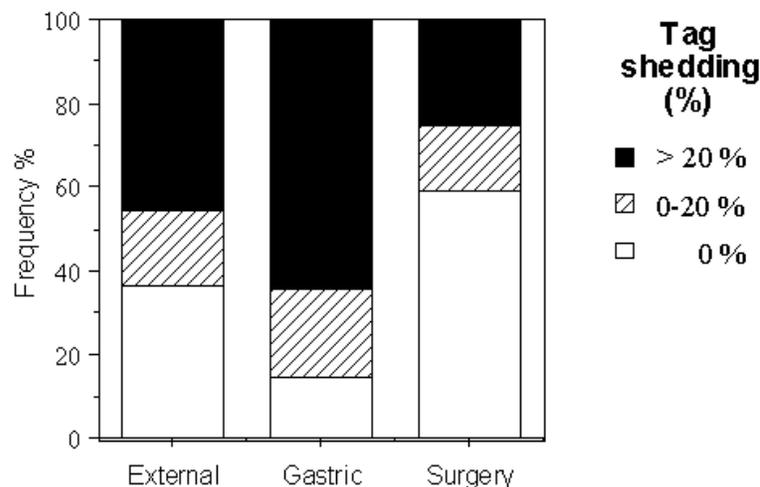


Figure 7.3. Proportion of telemetry studies reporting variable rates (0 %, < 20 %, 20 %) of tag shedding depending on attachment procedure.

a) Shedding of externally-attached tags

Externally-attached transmitters can be programmed to be shed by fish on purpose, by using absorbable attachment threads such as catgut, or by use of pop-up technology (Block et al 1998; Lutcavage et al. 1999). Tags fixed by non-absorbable threads are supposed to remain attached to the body of the fish, but shedding has been frequently reported (Figure 7.3), as exemplified by tags attached at the base of the anal fin of yellowtail, *Seriola quinqueriadata*, that were shed on average 8 days after tagging (Ichihara et al., 1972), or by tags attached dorso-laterally to lake whitefish, *Coregonus clupeaformis* (Bégout et al., 1998). The main causes invoked were untied knots (e.g. barbel, *Barbus barbus*, Baras, 1992; dace, *Leuciscus leuciscus*, Beaumont et al., 1996) or deep cuts in the dorsal musculature caused by attachment wires (e.g. lake whitefish, Bégout et al., 1998) as a result of drag. The use of cyanoacrylate adhesive at the time of tagging can secure knots. Attachment plates frequently used in side-saddle harnesses reduce the extent of cuts and subsequent shedding rates (e.g. < 5 % after three months in yellow perch, *Perca flavescens* and < 5 % after 37 d in black bass, *Micropterus salmoides*; Ross & McCormick, 1981; 0 % after 45 days in white perch, *Morone americana* and rainbow trout, *Oncorhynchus mykiss*, Mellas & Haynes, 1985). However, harnesses may cause erosion of scales and muscles in the long run and eventually promote microbial infection and death of tagged fish. Similarly, more secure knots may untie later, and possibly at different times, and thus cause the fish to drag the tag at the extremity of the attachment wire (Beaumont et al., 1996). This almost certainly modifies fish behaviour. Feasibility studies with externally-attached transmitters have rarely lasted more than 90 days, and it is thus uncertain whether tags may be retained for long periods, especially for side-saddle harnesses, which may strongly interfere with growth, and cause deep cuts to the fish musculature.

(b) Regurgitation and egestion of gastrically-inserted tags

Transmitters in bait that are voluntarily ingested by fish have never been reported to damage the digestive tract of the fish, whereas damage to the oesophagus was observed when transmitters were inserted with a plunger (McCleave & Horrall, 1970; Solomon & Storeton-West, 1983). Stomach-inserted or ingested transmitters may be lost through regurgitation (vomiting) or egestion (defecation). Regurgitation rates and delays between ingestion (or insertion) and regurgitation vary greatly, depending on the fish species and the relative size of the tag (Moser et al., 1990; Nielsen, 1992). Regurgitation rates generally increase as relative tag size increases (Nielsen, 1992). Small tags, in contrast, may be lost through egestion (Mortensen, 1990; Baras, 1992). Some species are known to regurgitate transmitters more frequently than others (Table 7.1). Recently, Marmulla & Ingendahl (1996) suggested that the mode of capture influenced the propensity of salmonids to regurgitate tags: sea trout captured with electric fishing in rivers regurgitated sooner and more frequently than those captured by netting.

Table 7.1. Fish species with high and low potential for retaining gastrically-inserted transmitters. (after Nielsen, 1992; adapted from Stasko & Pincock, 1977, and others).

Regurgitation unlikely	Regurgitation likely
<i>Alosa sapidissima</i> (American shad)	<i>Catostomus commersonni</i> (white sucker)
<i>Anguilla rostrata</i> (American eel)	<i>Esox lucius</i> (Northern pike)
<i>Ictalurus nebulosus</i> (brown bullhead)	<i>Gadus morhua</i> (Atlantic cod)
<i>Morone chrysops</i> (white bass)	<i>Katsuwonus pelamis</i> (skipjack tuna)
<i>Morone saxatilis</i> (striped bass)	<i>Oncorhynchus kisutch</i> (coho salmon)
<i>Oncorhynchus gorbuscha</i> (pink salmon)	<i>Oncorhynchus mykiss</i> (rainbow trout)
<i>Oncorhynchus keta</i> (chum salmon)	<i>Perca flavescens</i> (yellow perch)
<i>Oncorhynchus nerka</i> (sockeye salmon)	<i>Salmo salar</i> (Atlantic salmon)
<i>Oncorhynchus tshawytscha</i> (Chinook salmon)	<i>Salmo trutta</i> (brown trout)
<i>Salvelinus namaycush</i> (lake trout)	<i>Stizostedion canadense</i> (sauger)
<i>Thunnus thynnus</i> (bluefin tuna)	

c) Expulsion of surgically-implanted transmitters

By contrast with terrestrial vertebrates, fish maintain near-neutral buoyancy. They have not developed their abdominal region to cope with gravity effects like these induced by negatively-buoyant transmitters or tags, and this may account for the relatively frequent expulsion of implants by fish. Early implant exit may take place through the incision before healing is completed and is generally a consequence of loose suturing. Implants may be expelled later, either through the incision, through an intact part of the body wall, or through the intestine (channel catfish, *Ictalurus punctatus*, Summerfelt & Mosier, 1984; rainbow trout, *Onchorhynchus mykiss*, Chisholm & Hubert 1985; Lucas, 1989; Atlantic salmon smolts, *Salmo salar*, Moore *et al.*, 1990; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press). All three modes of exit share a common mechanism, which consists of the encapsulation of the implanted tag by proliferating granulation tissue consisting of collagen and myofibroblasts (Marty & Summerfelt, 1986, 1990). The contraction of myofibroblasts adds to the gravity pressure exerted by the transmitter over the fish tissue, and forces the implant through the route of least resistance. During the transintestinal expulsion process, the implant capsule adheres to at least two points of the intestinal peritoneum, as well as to the parietal peritoneum. The resulting rigidity interferes with the movements of the intestine during digestion and causes the dislocation of the muscular layer of the pyloric intestine, allowing the implant to pass into the lumen of the intestine and thence to be transported by reflex peristalsis to the anus.

Encapsulation is a classical body reaction and has been observed with all coatings assayed to date, and this suggests that the expulsion process is not specific to coating (Baras *et al.*, in press). Further, no anal or body wall exit was observed in some species like blue tilapias (*Oreochromis aureus*) which encapsulated implants almost systematically (Thoreau & Baras, 1997). It is worth emphasising that not all fish species encapsulate tags, and the propensity for expulsion is thus species-dependent, especially for transintestinal expulsion, which seems specific to siluriform species (channel catfish, *Ictalurus punctatus*, Marty & Summerfelt, 1986; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press). Factors that promote the expulsion of implanted tags include the position of the tag and tag:fish size ratios. Positioning the implant far from the incision, either through a plunger or using a shielded needle technique, minimises the risk of pressure over this weakened tissues and promotes long term retention of the implant (Ross & Kleiner, 1982; Baras & Westerloppe, in press). Incidence of rejection of transmitters through the body wall, or incision site, seems to increase with transmitter size (channel catfish, *Ictalurus punctatus*, Summerfelt & Mosier, 1984; Chisholm & Hubert, 1985; Marty & Summerfelt, 1986; rainbow trout, *Onchorhynchus mykiss*, Chisholm & Hubert 1985). Large transmitters are, however, less likely to enter the intestine and be expelled by peristalsis (Lucas, 1989; Baras & Westerloppe, in press). Bleeding during surgery favours the formation of clots and adhesions (Rosin, 1985) which are involved in the encapsulation and expulsion processes. Similarly, factors that promote the invasion of the body cavity by microbial organisms, such as external whip antennas of radio transmitters, or permanent suture materials, also increase the risk of expulsion (Baras *et al.*, in press). In this respect, braided suture filaments were recently shown to cause more frequent transintestinal expulsion in vundu catfish, *Heterobranchus unifilis*, than monofilaments (Baras & Westerloppe, in press), essentially because the former provide a larger surface for the settlement of micro-organisms than the latter. Prophylaxis and use of antibiotics may thus be extremely advantageous to minimise or prevent tag expulsion.

Although transmitter loss is undesirable scientifically, it should be noted that transmitter expulsion does not necessarily lead to subsequent mortality or morbidity (channel catfish, *Ictalurus punctatus*,

Marty & Summerfelt, 1986; rainbow trout, *Onchorhynchus mykiss*, Lucas, 1989; Atlantic salmon smolts, *Salmo salar*, Moore *et al.*, 1990; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press).

7.4.4. Infections and wounds

Fish with externally-attached and surgically-implanted transmitters may have infections and wounds at the attachment points and the incision (e. g. yellow perch, *Perca flavescens*, Ross & McCormick, 1981; white perch, *Morone americana*, Mellas & Haynes, 1985; barbel, *Barbus barbus*, Baras, 1992; bluegill, *Lepomis macrochirus*, Knights & Lasee 1996; European eel, *Anguilla anguilla*, Baras & Jeandrain, 1998). In freshwater, especially at higher temperatures, fungus infection may be a problem, especially for salmonids (rainbow trout, *Onchorhynchus mykiss*, Lucas 1989; Kaseloo *et al.*, 1992; Chinook salmon, *Onchorhynchus tshawytscha*, Adams *et al.*, 1998), but these infections are not specific to external wounds, since they were also observed in salmonids with gastrically-inserted transmitters, possibly as a consequence of handling (Solomon & Storeton-West, 1983). Infections are enhanced by the presence of permanent transcutaneous bodies (Roberts *et al.*, 1973) such as the threads of externally-attached transmitters, permanent suture material or externally trailing antennas of radio tags. Similar problems are also encountered frequently for gastrically-inserted transmitters with trailing antennas that cause abrasion of the mouth corner (e.g. Chinook salmon, *Onchorhynchus tshawytscha*, Martinelli *et al.*, 1998). Threads of external transmitters or heavy tags, as well as suture materials, can also cause deep cuts into the muscles and skin (yellowtail *Seriola quinqueradiata* Ichihara *et al.*, 1972; barbel, *Barbus barbus*, Baras, 1992; lake whitefish, *Coregonus clupeaformis*, Bégout *et al.*, 1998). These cuts promote further infection of the fish by microbial organisms (bluegill, *Lepomis macrochirus*, Knights & Lasee, 1996), or cause the tissue to become necrotic and prevent normal healing (rainbow trout, *Onchorhynchus mykiss*, Kaseloo *et al.*, 1992; bluegill, *Lepomis macrochirus*, Knights & Lasee, 1996; European eel, *Anguilla anguilla*, Baras & Jeandrain, 1998). Fast flowing environments, which increase the drag of externally-attached tags, can cause abrasion of the skin beneath the tag, or the foam pad on the side of the fish. These abrasions can eventually cause microbial invasion (white sucker, *Catostomus commersoni*, Lonsdale & Baxter, 1968; yellow perch, *Perca flavescens*, Ross & McCormick, 1981; hybrid bass Yeager, 1982; barbel, *Barbus barbus*, Baras, 1992; Atlantic cod, *Gadus morhua*, Thorsteinsson, 1995; sea bass, *Dicentrarchus labrax*, Claireaux & Lefrançois, 1998). The severity of wounds is often worse in cryptic or highly structured environments, in which externally-attached tags can become entangled in surrounding vegetation, or torn by rocky substrata (yellow perch, *Perca flavescens*, Ross & McCormick, 1981; Atlantic salmon, *Salmo salar* smolts, Nettles & Gloss, 1987; Chinook salmon, *Oncorhynchus tshawytscha*, Adams *et al.*, 1998).

Internally positioned transmitters can cause wounds too, either during inserting, or later, as a result of movements of the tag inside the fish. Plungers used to insert intragastric tags may damage the stomach or the oesophagus (cutthroat trout, *Oncorhynchus clarki*, McCleave & Horrall, 1970; sea trout, *Salmo trutta*, Solomon & Storeton-West, 1983). Scalpels may puncture viscera or ovaries, especially when making incisions laterally to the midventral line (grass carp, *Ctenopharyngodon idella*, Schramm & Black, 1984; Baras *et al.*, in press). Surgically-implanted transmitters may move inside the body cavity and cause various types of damage such as alterations to gonads (Chamberlain, 1979), internal haemorrhages (rock bass, *Ambloplites rupestris*, Bidgood, 1980; carp, *Cyprinus carpio*, Otis & Weber, 1982; Mortensen, 1990), bruised livers or erosion of the rectum (grass carp, *Ctenopharyngodon idella*, Schramm & Black, 1984), necrosis of the pelvic girdle

(bluegill, *Lepomis macrochirus*, Prince & Maughan, 1978) or rupture of the body wall or intestine prior to expulsion (channel catfish, *Ictalurus punctatus*, Marty & Summerfelt, 1986; rainbow trout, *Oncorhynchus mykiss*, Lucas, 1989; vundu catfish, *Heterobranchus longifilis*, Baras & Westerloppe, in press). Attempts have been made to suture implanted transmitters to the body wall in order to prevent movement inside the body cavity and consequent damage to viscera. However, these attempts have produced highly variable results depending on species. Petersen & Andersen (1985) succeeded while tagging Atlantic cod, *Gadus morhua*, whereas transmitters sutured to the body wall of channel catfish *Ictalurus punctatus* were almost systematically expelled (Marty & Summerfelt, 1986).

Most damage can be prevented, or alleviated, by tailoring the attachment procedure to the species of interest and prevailing environmental conditions. Adjustments include tag size, shape, length and coating, tag positioning, attachment threads (external tags), incision site and closing material (intraperitoneal tags), and use of appropriate prophylactic measures (see Summerfelt & Smith, 1990; Baras *et al.*, in press).

7.4.5. Effects on growth and feeding

Depressed growth rate, or weight loss of fish has been observed frequently after tagging, but with variable extent and duration, depending on fish species, life stage and attachment procedure. Growth is an integrating variable of fish physiology and behaviour, and impaired growth may thus be the consequence of habitat change, depressed mobility or competitive ability, difficulties in recovering buoyancy, change of social status, increased energy expenditures or reduced appetite.

The degree of stomach fullness is a well-known factor that regulates the appetite of fishes. Feeding can be terminated by a full stomach (Toates, 1981; Jobling, 1994) and gastrically-inserted tags may induce similar reactions. The problem does not arise with adult salmonids and other species that do not feed during spawning migrations. Tags affect food intake in proportion to the tag:fish weight ratio, although it seems likely that this effect is governed by the relative volumes of tag and stomach. Moser *et al.* (1990) observed that tag ratios less than 4.5 % did not affect feeding and growth of juvenile coho salmon, *Oncorhynchus kisutch* whereas higher ratios (4.5-14.5 %) reduced the feeding rate. Similarly, Armstrong and Rawlings (1993) reported that Atlantic salmon (*Salmo salar*) parr did not feed after the insertion of transmitters into their stomachs. Adams *et al.* (1998) and Martinelli *et al.* (1998) observed that gastrically-inserted transmitters averaging 4 and 6% of the body weight of juvenile Chinook salmon, *Oncorhynchus tshawytscha*, impaired their growth over longer periods than tags inserted into the peritoneum. However, not all species seem to be affected in the same way, since the food intake of Atlantic cod, *Gadus morhua*, was not modified after gastric-insertion of transmitters (Reference needed). Whether abrasion of the corner of the mouth, which is frequently observed in fish tagged with transmitters involving the external antenna trailing from the mouth (e.g. Chinook salmon, *Oncorhynchus tshawytscha*, Martinelli *et al.*, 1998), affects the feeding rate or growth of the fish, is uncertain.

No long term effects on feeding and growth have been found in studies with surgically-implanted transmitters in muskellunge (*Esox masquinongy*; Crossman, 1977), channel catfish (*Ictalurus punctatus*; Summerfelt & Mosier, 1984), Colorado squawfish (*Ptychocheilus lucius*; Tyus, 1988), razorback sucker (*Xyrauchen texanus*; Tyus, 1988), juvenile Atlantic salmon (Moore *et al.*, 1990) and rainbow trout (Lucas, 1989, Martin *et al.*, 1995). However, studies where growth was investigated at shorter time intervals provided evidence that the growth of surgically-tagged barbel

(*Barbus barbus*; Baras, 1992), vundu catfish (*Heterobranchus longifilis*, Baras & Westerloppe, in press) or blue tilapia (*Oreochromis aureus*; Thoreau & Baras, 1997) was impaired over the first few post-tagging days, but was then compensated for by higher than normal growth rates. Growth rate returned to normal again when the surgical incisions had healed. Factors invoked included partly excessive tag ratios that restricted access to food resources, or feeding subordinated to untagged individuals that appeared dominant at feeding time (bluegill *Lepomis macrochirus*, Knights & Lasee 1996). During the transintestinal expulsion process in catfishes, tags may also cause a transient blockage of food, of which the duration is uncertain, but is apparently long enough to depress the growth of the fish.

The effects of external tags on feeding and growth rate have also been investigated, but essentially during short or mid-term feasibility studies. No effects were found in yellow perch (*Perca flavescens*; Ross & McCormick, 1981), dace (*Leuciscus leuciscus*; Beaumont *et al.*, 1996) or lake whitefish (*Coregonus clupeaformis*; Bégout-Anras *et al.*, 1998), whereas externally-tagged largemouth bass (*Micropterus salmoides*) showed lower predation rates on minnows (Ross & McCormick, 1981), and barbel (*Barbus barbus*) carrying external dummy tags lost weight over several weeks after tagging (Baras, 1992). Similarly, the feeding rates and growth in parr of Atlantic salmon (*Salmo salar*) was affected by external tagging, and growth impairment was proportional to the tag ratio (Greenstreet & Morgan, 1989). In contrast to intraperitoneally-implanted transmitters, the effects of external tags on growth and feeding may be progressive and increase in the long run, essentially because of permanent wounds, and generally deeper cuts to the musculature as time goes by. Side-saddle harnesses are also deemed to interfere mechanically with the growth of the fish but no study has evaluated this problem over long periods.

7.4.6. Effects of tags on behaviour

The effects of tags and tagging procedure on fish behaviour or physiology have been relatively poorly documented, essentially because these aspects have rarely been investigated during feasibility studies (see Figure 7.1). Reasons for this include the difficulty of measuring physiological variables accurately in live fish without causing additional interference, and the discrepancy between experimental environments used in feasibility studies and wild environments. Furthermore, changes in behaviour can be more discrete and last for shorter periods of time, and thus be far less obvious to detect than mortality, tag shedding or reduced growth.

(a) Buoyancy and posture

With few exceptions (e.g. tunas or catfishes), teleost fish maintain reduced body density by adjusting the volume of their swim bladder. Many fish with swim bladders are negatively buoyant over much of the water column, only approaching neutral buoyancy at the top of their vertical range (Blaxter & Tytler, 1978; Harden Jones & Scholes, 1985; Arnold & Greer Walker, 1992). The swim bladder is said to have a volume of about 5 % of fish volume in marine fishes, and about 7 % in freshwater fishes, though these are theoretical values and real data are much more variable. More importantly, the swim bladder has an adjustment capacity of about 25 % (Alexander, 1966; Bone & Marshall 1982). This adjustment capacity permits the fish to cope with increased mass, such as that caused by negatively-buoyant eggs or tags. Physostomatous fish such as salmonids or anguillids possess a connection between the swim bladder and the gut, and can refill their swim bladder by swallowing air. The connection is absent in the vast majority of teleosts (physoclistous fish), in which gas exchange takes place via the *rete mirabile* (Bone & Marshall, 1982).

This anatomical difference implies that physostomes can regain near-neutral buoyancy more rapidly than physoclists after attachment of a negatively-buoyant transmitter or DST, provided they can access the surface (e.g. Atlantic salmon, *Salmo salar*, Fried *et al.*, 1976). Physoclistous percids remain on the bottom until sufficient gas is secreted, whereas cichlids or centrarchids like the bluegill (*Lepomis macrochirus*) increase their fin beat frequency to create the upward force necessary to reach shallow depths where they can achieve neutral buoyancy (Gallepp & Magnuson, 1972). Similarly, blue tilapia *Oreochromis aureus* take about 72 hours to compensate for the negative buoyancy and slight postural disequilibrium caused by implantation of a transmitter which adds 0.9 % to their body mass (Thoreau & Baras, 1997). Swimming compensation may also take place in physostomatous fish denied access to the surface (Fried *et al.*, 1976), and in negatively-buoyant fish like scombrids or thunnids, which swim continuously to avoid sinking and for which adding weight implies faster swimming.

Tagging thus imposes temporary or permanent constraints on fish bioenergetics, of which the energetic cost has rarely been quantified, but is presumably directly proportional to the tag:fish weight ratio. This accounts partly for the observation that most fish carrying tags representing more than 1.75-2.00 % of their body weight in water show deviant behaviour subsequent to tagging, whereas minimal or zero effects are observed for lower ratios (e.g. McCleave & Stred, 1975; Greenstreet & Morgan, 1989; Moser *et al.*, 1990; Kaseloo *et al.*, 1992; Voegeli *et al.*, 1998). More adverse effects of capture and release procedures can theoretically take place when fish are captured in deep water and transported to the surface for tagging, as this rapid change of depth can damage the swim bladder (see Chapter 5).

(b) Swimming performance and energetic expense

As mentioned earlier, negative buoyancy induced by tagging may cause the fish to increase its fin beat frequency to compensate for added mass, regardless of the attachment procedure. However, additional specific adverse effects may originate from the procedure itself. Externally-attached tags are usually positioned further from the centre of gravity of the fish than internally positioned tags. Because of this they are more prone to cause permanent or temporary postural disequilibrium and irregular swimming (e.g. Atlantic salmon *Salmo salar*, Thorpe, 1981; largemouth bass *Micropterus salmoides*, Mellas & Haynes, 1985; dace *Leuciscus leuciscus*, Beaumont *et al.*, 1996). Drag resistance of externally-attached tags varies depending on transmitter bulk and shape.

Swimming performance may be affected by the presence of a transmitter, which is especially important to consider when dealing with migratory species, such as salmonids, and active pelagic species, such as scombrids. Drag resistance of externally-attached transmitters is the most obvious cause of reduced swimming capacity, but large internal transmitters may inhibit swimming movements, reducing available power. Other effects of transmitters that reduce the health of the fish and/or increase the energy demand, will also combine to affect swimming performance.

Externally-tagged rainbow trout have been shown to exhibit lower exhaustion times than other tagged groups or control fish (Mellas & Haynes 1985). In another study of rainbow trout, two types of externally-attached transmitters raised both tail beat frequency (TBF) and opercular beat rate (OBR), but a transmitter consisting of two packages mounted symmetrically on either side of the body affected TBF and OBR least (Lewis & Muntz 1984). In a study of Atlantic salmon smolts, critical swimming speeds were lower in fish with external transmitters (McCleave & Stred 1975). Drag measurements of external transmitters in a flume indicated that the extra power output required for tagged plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*) to maintain the same steady

speed as untagged fish was between 3 and 5 %, which in this study was considered negligible (Arnold & Holford 1978). In a field study of adult chinook salmon (*Oncorhynchus tshawytscha*), upstream migration in a river was successful in externally-tagged fish, which migrated at the same speed as control fish. In contrast, most of the fish with surgically-implanted transmitters were not able to pass a dam, and eventually migrated downstream (Gray & Haynes 1979). No effects of the transmitters on swimming performance were detected in swimming tests of juvenile Atlantic salmon with surgically-implanted transmitters, white perch (*Morone americana*) with surgically-implanted, externally-attached and gastrically-inserted transmitters, and rainbow trout (*Oncorhynchus mykiss*) with surgically-implanted and stomach-inserted transmitters (Mellas & Haynes 1985, Moore *et al.*, 1990).

Studies dealing with swimming performance of tagged fish demonstrate that the effects vary considerably. Swimming performance seems least affected when transmitter size and volume are as small as possible in proportion to fish size (e. g. McCleave & Stred 1975).

(c) Effect on social behaviour and interactions between species.

The effect of tagging or tag presence on predation risk has rarely been investigated in feasibility or field studies. Because of the difficulty in recovering neutral buoyancy, or because of reduced swimming capacities, fish tagged with electronic tags may be more vulnerable to predation than untagged fish (Jolley & Irby, 1979; Ross & McCormick, 1981; Eiler, 1990). External tags may also make tagged fish more easily detected by predators, and it is thus recommended that external transmitters are camouflaged to reduce their visibility (Ross & McCormick, 1981). Similarly, handling or tagging procedures may affect the social status of the fish. Surgically-tagged Guadeloupe bass (*Micropterus treculi*) showed less social tendencies than untagged fish (Manns & Whiteside, 1979; Manns, 1981), and externally-tagged yellowtail (*Seriola quinqueradiata*) showed depressed social behaviour over the first hour after tagging (Ichihara *et al.*, 1972). In other circumstances, tagging did not modify shoaling or schooling (e.g. Baras, 1997). With respect to species exhibiting territorial behaviour or social hierarchy, occasional changes of social status were observed in rainbow trout (*Oncorhynchus mykiss*) carrying tags in their stomach (Mellas & Haynes, 1985), whereas surgery was not enough to cause reversal of a well established hierarchy, either in brown trout (*Salmo trutta*; Baras *et al.*, in prep.) or rainbow trout (*Oncorhynchus mykiss*, Swanberg & Geist, 1997). However, similar status changes were also seen in fish that had only been handled, suggesting that this adverse effect did not originate from tagging, but from the capture and handling procedure (Baras *et al.*, in prep.). It is strongly suggested that such adverse effects on fish behaviour must also be considered when tagging fish with conventional tags or PIT tags.

Considering the various adverse effects of tagging and their dynamics, the risk of predation or change of social status is highest during the post-tagging hours or days for all attachment procedures, then vanishes when wounds have healed. Exceptions to this rule of thumb are mainly concerned with external transmitters, for which adverse effects can cumulate over time. This applies particularly to spawning behaviour, and it is generally recommended that fish are not tagged during the reproductive period (Winter, 1996). Fish are deemed to be more delicate at this time (Økland *et al.*, 1996) and there is a higher risk of damaging the enlarged gonads of females when implanting tags in the body cavity (Bidgood, 1980; Schramm & Black, 1984). However, adverse effects of tagging mature fish are not systematically observed and some species spawn successfully less than one week after abdominal surgery and transmitter implantation (Baras, 1995). Similarly, most studies where the gonadal development of fish with surgically-implanted tags has been evaluated show little or no

difference from controls (Moore *et al.*, 1990, 1994; Martin *et al.*, 1995; see parallel with PIT tags in Baras *et al.*, in press). There may even be advantages in tagging mature individuals of species like the vundu catfish, *Heterobranchus longifilis*, in which enlarged gonads may prevent transintestinal expulsion of tags (Baras & Westerloppe, in press).

(d) Mobility and habitat selection

There are a few studies of the effects of tags on mobility and habitat selection in artificial rivers (e.g. brown trout, *Salmo trutta*, Baras *et al.*, in prep.), or culture tanks (e.g. blue tilapia, *Oreochromis aureus*, Thoreau & Baras, 1997). Most tag-induced biases have, however, been reported from field studies. Irregular swimming, erratic movements and apparent disruption of surface avoidance behaviour have been reported in several species (Guadeloupe bass, *Micropterus treculi*, Manns & Whiteside, 1979; largemouth bass, *Micropterus salmoides*, Mesing & Wicker, 1986). Hypoactivity of newly tagged fish is most frequent (e.g. rainbow trout, *Oncorhynchus mykiss*, Zimmermann, 1980; blue tilapia, *Oreochromis aureus*, Thoreau & Baras, 1997), as well as increased downstream movements of upstream migrants (Chinook salmon, *Oncorhynchus tshawytscha*, Haynes & Gray, 1979). However, post-release hyperactivity has been observed too (Atlantic cod, *Gadus morhua*, Hawkins *et al.*, 1974; Lake whitefish, *Coregonus clupeaformis*, Bégout-Anras *et al.*, 1998). Further, both hypo- and hyperactivity have been observed in the same species (Thoreau & Baras, 1997), and this makes it difficult to determine whether these were just normal changes in the activity level of the fish, or actual perturbations resulting from the tagging procedure. Similarly, both upstream and downstream movements were observed in sick brown trout, *Salmo trutta*, that died eventually, and long downstream movements were observed in healthy individuals (M. Ovidio, unpublished data).

This variability considerably limits the relevance of behavioural criteria, essentially because the behaviour of the fish prior to tagging is generally unknown. Hence it is suggested (Lagardère *et al.*, 1996; Baras *et al.*, in press) that these criteria would be best used within a framework of individual modes, for an *a posteriori* determination of when the fish stopped behaving normally.

(e) Additional perturbations of behaviour

The use of electronic tags in fisheries is deemed to minimise the subsequent stress of recapture that is frequently encountered in conventional tagging studies. However, radio or acoustic telemetry frequently implies that the fish is tracked from the banks of a river, or from a tracking boat in lakes or at sea, and this may cause temporary perturbations of fish behaviour. Vibrations on river banks during tracking can cause fish to move away from the noise source, or to dive in deeper water (Baras, unpublished). Similar behaviour was reported for European eels (*Anguilla anguilla*); these do not change swimming direction, but dive to greater depth when a boat approaches within 10 m, then regain their original depth after the boat has passed (Westerberg, 1983). Boat engines are extremely noisy and can be detected at distances of hundreds of meters by several fish species, including Atlantic cod *Gadus morhua* (Stasko & Buerkle, 1975). Whether all fish change their mobility pattern at the approach of a boat is uncertain. Stasko & Pincock (1977) stated that pink salmon (*Oncorhynchus gorbuscha*), Chinook salmon (*O. tshawytscha*), American eel (*Anguilla rostrata*), white bass (*Morone chrysops*) and largemouth bass (*Micropterus salmoides*) were apparently not affected, while reactions had been reported frequently in dusky shark (*Carcharhinus obscurus*), white marlin (*Tetrapturus albidus*) and in some cases in sockeye salmon (*O. nerka*) and Atlantic

salmon (*Salmo salar*). Avoidance reactions of marine fish to research vessels and fishing gear are discussed in some detail in Miston (1995)

7.4.7. Effects of tags on physiology

Although the physiology of newly tagged fish has rarely been investigated, one aspect of this problem has already been addressed indirectly in section 7.3.3.f., which deals with the physiological changes (i.e. increased gas exchange or increased rates of fin movement) that may be needed to compensate for the added mass of the tag.

Surgically-tagged fish with open incisions may experience difficulty in maintaining their osmotic balance, and their physiology may thus be affected for a variable period, whose length will depend on the capacity of the fish to repair tissue. This period is likely to last at least until the incision is filled with connective tissue (2 days to several weeks, depending on species, age and temperature; see Anderson & Roberts, 1975; Baras *et al.*, in press). It should be complete once the epidermis has been reconstituted over the incision area. However, these aspects have never been investigated in detail, and it is also uncertain whether quicker ways to close the incision, such as use of cyanoacrylate adhesives, minimise the problem (Nemetz & MacMillan, 1988; Petering & Johnson, 1991; Baras & Jeandrain, 1998). Similarly, the effects of chronic lesions caused by the threads of external tags on osmotic balance are unknown.

There is little doubt that infections, haemorrhages or damage to organs due to erosion by the tag, or the tag expulsion mechanism, affect fish physiology too, but the extent of these perturbations has rarely been measured during tagging feasibility studies. Martinelli *et al.* (1998) provided evidence for reduced levels of plasma proteins in newly tagged Chinook salmon (*Oncorhynchus tshawytscha*) that lasted for at least 5 days in surgically-tagged fish, and at least 21 days in fish carrying transmitters in their stomachs. These changes were deemed to reflect reduced food intake. Claireaux and Lefrançois (1998) measured metabolic rates of externally-tagged Atlantic cod (*Gadus morhua*) and sea bass (*Dicentrarchus labrax*) and found that these were substantially higher than in untagged fish, although they estimated that the impact of tag carrying was low with respect to the metabolic capacities of these two species.

7.4.8. Effects of PIT tags

Because of their small size (11 x 2.2 mm in diameter, 70 mg in the air and 40 mg in water), there is a low probability that PIT tags cause a major interference with fish life processes (Nielsen, 1992), and this is indeed the case in husbandry management programmes where the technique is used (Jenkins & Smith, 1990; Poncin *et al.*, 1990). Short term effects of PIT tagging have been noticed while tagging broodstock, but these are mainly a result of capture and handling (Baras & Westerloppe, in press).

However, precisely because of their small size, PIT tags can be applied to small juvenile fish (Prentice *et al.*, 1990; Peterson *et al.*, 1994; Ombredanne *et al.*, 1998), which may thus be confronted with problems similar to those encountered in telemetry studies with adult fish, where transmitters are implanted into the body cavity. These include difficulties in buoyancy compensation, reduced access to food and slower growth over the first post-tagging days when using tag ratios above 3 % in the air (Nile tilapia, *Oreochromis niloticus*; Baras *et al.*, in press; perch, *Perca fluviatilis*; Baras *et*

al., submitted). Similar but less severe effects were noticed in fish with lower tag ratios (Baras *et al.*, *op cit.*; Baras & Westerloppe, in press). Ombredanne *et al.* (1998) also reported depressed growth of brown trout (*Salmo trutta*) parr after PIT tagging, but the extent of growth depression was comparable with that observed after adipose fin clipping alone. As for most other tags implanted surgically, normal growth resumes when the incision has healed. Healing is usually achieved in less than 14 days (salmonids; Prentice *et al.*, 1990), and sometimes as fast as 7 days (catfishes; Baras & Westerloppe, in press), either because the incision is small compared with those used for telemetry tags, or because the fish are younger and have greater capacity for wound repair. In contrast to salmonids, the healing rate in small juvenile perch and tilapia is faster when the PIT tag is inserted manually through an incision made with a scalpel than when using conventional injectors (Baras *et al.*, in press). The latter procedure also causes much higher mortality rates than the former, and this contrasts too with young salmonids, for which injectors are usually efficient and innocuous. The relative inadequacy of injectors in tilapia or perch smaller than 10 g is due to the difficulty of controlling the penetration of the hypodermic syringe following piercing of the body wall. This is much more rigid than in salmonids, for which the injector was originally developed.

PIT tags are encapsulated in inert glass, which has few adverse effects on fish tissues, even several years after implantation. Plastic tips covering PIT tags further limit their propensity to migrate through muscular tissues, causing further damage. Probably for these reasons, the retention of PIT tags is usually extremely high (92-96 % in juvenile snapper, *Pagrus auratus*, Quartaro & Bell, 1992; 96.6 % in juvenile *Salmo trutta*, Ombredanne *et al.*, 1998; 99-100 % in Chinook salmon, *Oncorhynchus tshawytscha*, Prentice *et al.*, 1990; 100 % in largemouth bass, *Micropterus salmoides*, Harvey & Campbell, 1989). By analogy with observations in studies where sutured and non sutured incisions were evaluated (Baras *et al.*, in press; Baras *et al.*, submitted), it is likely that most tags were lost via the incision before the wound had healed. As observed for telemetry transmitters, PIT tags remained free in the body cavity of some species (Salmonids: Prentice *et al.*, 1990), whereas they frequently became encapsulated in others (Cichlids; Baras *et al.*, submitted a; Percids; Baras *et al.*, submitted b; Clariids; Baras & Westerloppe, in press). Though encapsulation was frequent in these species, no single tag expulsion was observed in juvenile tilapia or perch, at least when the incision had been closed by a single stitch. Some catfishes, however, expelled the tag through the intestine, as observed for electronic tags in adults (Baras & Westerloppe, in press).

Effects of PIT tags on physiology and behaviour have rarely been investigated. Jenkins & Smith (1990) found no adverse effect of PIT tagging on spawning in breeders of red drum (*Sciaenops ocellatus*) and striped bass (*Morone saxatilis*), and PIT tagging juvenile tilapia did not prevent their sexual maturation and breeding (Baras *et al.*, in press). Similarly, no difference was observed between the development of gonads or accumulation of abdominal lipid reserves in PIT tagged and untagged juvenile perch (Baras *et al.*, submitted b). No effect on swimming stamina or stride efficiency was found in PIT tagged juvenile Chinook salmon and rainbow trout (Prentice *et al.*, 1990), but signs of negative buoyancy were observed in juvenile perch and tilapias where tag ratios were higher than 3 %.

7.5. CONCLUSIONS

- 1) Tagging fish with electronic tags can generate numerous biases, the extent of which and duration of varies between species and environments. However, successes have been associated with attachment procedures tailored to the species of interest during the course of feasibility studies.

- 2) Scientists using electronic tags are increasingly selecting surgical techniques, mainly because adverse effects decrease over time. Surgery, however, involves longer training and more practice than is required for other attachment procedures.
- 3) In all tagging studies, attention should be paid to the size of the tag since excessive added weight is the most widely cited adverse bias. The tag:fish weight ratio should be kept low and drag, too, should be minimised when external tags are used. Research programmes should also be tailored to the capacities of the fish instead of imposing constraints that cannot be overcome by the fish, except after an adaptive process, whose duration exceeds that of the study.
- 4) Fish species have anatomical, physiological and behavioural peculiarities that make them unique, and it is thus worthwhile designing a feasibility study before implementing any field research, both for animal welfare reasons and reliability of results.
- 5) Increasing attention should be dedicated to lesser studied factors, such as attachment threads, closing material, tag shape and coating, pre- and post-operative care and confinement, since these may condition the actual success of tagging, and duration of post-tagging perturbation.
- 6) Identifying the duration of the post-operative perturbation is a sensible goal in any feasibility study, especially since electronic tags can now be programmed to transmit or collect data after delayed starts. DSTs can also be used to record post-operative effects, and thus observe directly how long the process lasts.

7.6. EFFECTS OF TAGS ON ORGANISMS OTHER THAN FISH

An exhaustive review of this topic is outside the remit of CATAG, but a few points are worth making. Tagging of marine mammals and birds (particularly seals and penguins) is common. Metal flipper tags are usually used for identification and are attached without anaesthetic. Tagging by hot-iron branding is still extensively used (e.g. on elephant seal pups; Feydak, personal communication). Though frowned upon ethically or for reasons of animal welfare, it is an extremely useful technique because the brands are readable after many years, whereas metal tags are lost. Satellite tags have been applied to both seals and penguins and are usually attached to fur or plumage by adhesives. This involves anaesthesia in seals (because they cannot be conveniently and safely immobilised in any other way). This anaesthesia may involve double administration of anaesthetics, first by darting to capture the animal concerned, secondly by administration of spinal anaesthesia during the tag attachment process. Care has to be taken to ensure that darted animals do not reach the water before capture; drowning is a significant risk.

Marine turtles have largely been tagged with flipper tags for identification. Tag loss rates are high and holes in flippers made during tagging may be susceptible to fungal infection. Satellite tags have also been attached to sea turtles. These cause minimal problems for the hard-shelled green, loggerhead, ridley and hawksbill turtles, other than increasing drag resistance (and presumably energy expenditure), but there are special problems with the large leatherback turtle *Dermochelys coriacea*. Satellite tags cannot be attached by adhesives because of the leathery, oily nature of the carapace and plastron. Early trials with towed tags, or tags attached by webbing harnesses failed

with some mortalities (unacceptable in an endangered, protected species). Current satellite tagging with this species involves the fitment of plastic-protected harnesses with biodegradable portions that allow the harness to be lost after some weeks or months.

Crustaceans have been tagged at least since the 1930s. Originally, tags were attached to crabs and lobsters to establish distances of migration and metal (later plastic) tags were simply wired through holes in the shell. These holes often enlarged and showed signs of infection. The main problem for crustacean tagging is to attach a tag that remains on the animal when it molts. Wired tags had to be very carefully placed along molt lines on the carapace to achieve this. Modern tags (lobster tags, spaghetti tags, streamer tags) are generally attached to the animal by piercing muscles, often with barbed anchors, or passing tags through the abdominal musculature from one side to the other (See Chapter 4 for more detail). Access is through arthroal membranes, not the hard shell. If this is done effectively, the tags usually survive molting. However, there are some reports of growth after molt being distorted by poorly-placed tags. No welfare problems have been reported from lobster stock enhancement programmes involving injection of coded-wire tags into the tail musculature of juvenile lobsters.

7.7. REQUIREMENTS AND RECOMMENDATIONS

All tagging procedures should aim at minimising short-term pain and stress to fish, and should avoid, as far as possible, causing long-term deterioration in health.

Planning of new tagging trials on familiar species should always involve full consideration of existing data on procedures, to ensure that mortalities, ill-health and tag losses are minimised. Laboratory feasibility studies to establish effective procedures on new species should ideally precede full field trials.

Fish tagging practitioners should all be required to undergo training. Current legislation often requires experimentation license holders to undergo generalised training in the legality of various procedures and holding techniques, but surgical procedures on fish are very different from those used on terrestrial mammals.

Anaesthesia should be used to minimise pain and trauma, save in circumstances where anaesthesia itself is more detrimental to fish.

All efforts should be made to avoid chemical residues associated with the tagging process reaching the human food chain.

Discussions amongst CATAG participants suggest that low temperatures may be effective in having an anaesthetic-like effect, at least in some fish species. Where such procedures are legal, it has sometimes been found that survival of surgical procedures is better when fish are kept cold during surgery than if they are anaesthetised. It is recommended that research (including neurophysiological investigations) be carried out to evaluate whether lowered environmental temperature is a humane approach to support of tagging operations involving surgery. It is appreciated that such research would have to encompass warm-temperate fish, as well as cold-water species. In addition, the long-term consequences of cold-exposure would also require study.

7.8. REFERENCES

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7.9. APPENDIX I.

Summary of main results from studies dealing with effects of ultrasonic and radio transmitters on fish. (http://www.hafro.is/catag/f-health&welfare/studies-res_2.htm)

7.10. APPENDIX II. Downloadable information sheets

Description of the ideal anaesthetics

(modified after Marking & Meyer, 1985, in Summerfelt & Smith, 1990)

- a) Induction < 15 min, and ideally < 3 min
- b) Recovery < 5 min
- c) No toxicity for fish, and large tolerance margins for concentration
- d) No persisting effect on fish physiology and behaviour
- e) Fast excretion and/or catabolism, leaving no residues in fish tissues
- f) No acclimatory or cumulative effects
- g) No danger for operators
- d) Easy preparation
- i) Low Cost

**Indicative list of the cost (1998 levels) of the main anaesthetics used in fish tagging.
The cost of 1 litre of anaesthetic solution is calculated for cyprinid species at 15°C.**

Compound	Presentation	Cost (ECU, VAT excl.)	Cost per litre of anaesthetic solution (ECU, VAT excl.)
Amobarbital	Powder	312 / 50 g	0.94
Benzocaine	Crystals	91 / kg	0.01
2-phenoxy-ethanol	Liquid	25 / l	0.01
Quinaldine (90 %)	Liquid	96 / l	0.03
Quinaldine sulphate	Powder	114 / 25 g	0.11
Tricaine	Crystals	180 / 100 g	0.18
Xylocaine (lidocaine)	Powder, Crystals	111 / 250 g	0.11

Tentative key for decision making when choosing between anaesthetics for fish handling and tagging

Criteria	
1.	Fish destined (C) or not destined (N) for consumption by humans
2.	Deep anaesthesia required (D) or sedation only (S, e.g. weighing)
3.	Natural environments (M), or experimental facilities, aquaculture (A)
4.	High or low volume of anaesthetic solution requested (H / L)
Anaesthetics, in decreasing order of preference	
(*) = expensive, (#) = difficult to implement	
CDMH:	Tricaine (stock solution)
CDML:	Tricaine (stock solution) (*), Hypothermia(#)
CDAH:	Tricaine (crystals), Hypothermia
CDAL:	Hypothermia, Tricaine (crystals) (*)
CSMH:	Tricaine (stock solution), Carbon dioxide (#), Electrical anaesthesia (DC)
CSML:	Electrical anaesthesia (DC), Tricaine (solution stock) (*), Carbon dioxide (#)
CSAH:	Tricaine (crystals), Carbon dioxide
CSAL:	Electrical anaesthesia (DC), Carbon dioxide, Tricaine (crystals) (*)
NDMH:	2-phenoxy-ethanol, Hypothermia, Tricaine (stock solution)
NDML:	2-phenoxy-ethanol, Hypothermia, Tricaine (stock solution) (*)
NDAH:	Tricaine (crystals), 2-phenoxy-ethanol, Hypothermia
NDAL:	2-phenoxy-ethanol, Hypothermia, Tricaine (crystals) (*)
NSMH:	2-phenoxy-ethanol, Quinaldine sulphate, Tricaine (stock solution), Carbon dioxide, Electrical anaesthesia (DC)

NSML:	Electrical anaesthesia (DC), 2-phenoxy-ethanol, Quinaldine sulphate, Tricaine (stock solution) (*), Carbon dioxide (#)
NSAH:	2-phenoxy-ethanol, Quinaldine sulphate, Tricaine (stock solution), Carbon dioxide, Electrical anaesthesia (DC)
NSAL:	Electrical anaesthesia (DC), Carbon dioxide, 2-phenoxy-ethanol, Quinaldine sulphate

Typical concentrations of tricaine and 2-phenoxy-ethanol recommended for deep anaesthesia

(for deep sedation about half the dose is required)

C (cold water, 5-15°C), T (temperate water, 10-25°C), W (warm water > 25°C)

Species	Family	Env.	Tricaine (mg / l)	2-phenoxy-ethanol (ml / l)
<i>Salmo salar</i> (Atlantic salmon)	Salmonidae	C	25	0.20-0.40
<i>Oncorhynchus sp.</i> (Pacific salmon)	Salmonidae	C	40-60	0.20-0.30
<i>Gadus morhua</i> (cod)	Gadidae	C	50	??
<i>Thymallus thymallus</i> (grayling)	Thymallidae	C	50-70	0.25
<i>Oncorhynchus mykiss</i> (rainbow trout)	Salmonidae	C	60	0.30-0.40
<i>Salmo trutta</i> (brown trout)	Salmonidae	C	50-75	0.20-0.30
<i>Brycon moorei</i> (dorada)	Characidae	W	80-100	0.40
<i>Perca fluviatilis</i> (Eurasian perch)	Percidae	T	90	0.40
<i>Oreochromis niloticus</i> (Nile tilapia)	Cichlidae	W	100	0.40
<i>Piaractus brachypomus</i> (colossoma)	Serrasalminidae	W	100	0.40
<i>Prochilodus magdalenae</i> (bocachico)	Curimatidae	W	100	0.40
<i>Barbus barbus</i> (barbel)	Cyprinidae	T	100	0.40
<i>Leuciscus cephalus</i> (chub)	Cyprinidae	T	100	0.40
<i>Morone saxatilis</i> (striped bass)	Percichthyidae	T	100	??
<i>Cyprinus carpio</i> (common carp)	Cyprinidae	T-W	100-150	0.35-0.60
<i>Lepomis macrochirus</i> (bluegill)	Centrarchidae	T-W	150	??

<i>Carassius auratus</i> (goldfish)	Cyprinidae	T-W	150-250	> 0.40
<i>Clarias gariepinus</i> (catfish)	Clariidae	W	120-300	0.40-0.60
<i>Anguilla anguilla</i> (European eel)	Anguillidae	C-T	250-500	0.80-1.00

Use of anaesthetics in fish telemetry tagging procedures

<u>Usual name</u>	Tricaine	<u>Exact name</u>	3-amino benzoic acid ethyl ester methanesulphonate
<u>Synonyms:</u>	Tricaine methanesulphonate, salt of methanesulphonate, metacaine, MS-222™, Finquel™		
<u>Conditioning:</u>	<ul style="list-style-type: none"> - crystals highly soluble in water (1 g / 9 ml) - stock solutions short term) 		
<u>Conservation:</u>	<ul style="list-style-type: none"> - Opaque bottle, stored at low temperature (crystals) - Freezing (stock solution) 		
<u>Typical concentrations:</u>	Salmonids	25-60 mg / l	
	Cyprinids	80-150 mg / l	
	Cichlids, Characids	± 100 mg / l	
	Catfishes	100-250 mg / l	
	Eels	_ 250 mg / l	
<u>Drawbacks:</u>	<ul style="list-style-type: none"> - Affects the olfactory epithelium (channel catfish) - Acid solution, which can affect the motility of spermatozoa, and cause respiratory stress - High cost 		
<u>Toxicity</u>	<ul style="list-style-type: none"> - non mutagenic - No specific toxicity at the concentrations above 		
<u>Permanence, legal aspects:</u>	<ul style="list-style-type: none"> - Insignificant residues after 24 h - 21-d delay between anaesthesia and consumption (FDA) 		

<u>Suggestions</u>	<ul style="list-style-type: none"> - Add sodium bicarbonate (NaHCO₃) before anaesthesia to buffer the anaesthetic solution (about 250 mg de NaHCO₃ for 100 mg of tricaine) - Do not buffer a stock solution before storage(inactivation)
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<u>Usual name</u>	2-phenoxy-éthanol	<u>Exact name</u>	1-hydroxy-2-phenoxyetane
<u>Synonyms:</u>	Ethylene glycol monophenyl ether, phenoxetol, phenoxethol, beta-hydroxyethyl phenyl ether, phenyl cellosolve		
<u>Conditioning:</u>	- Dense (1.1 g / l), transparent liquid, with low solubility in water (27 g / l) but high solubility in alcohol		
<u>Conservation:</u>	- Opaque bottle		
	<u>Typical concentrations:</u>	Salmonids	0.2-0.4 ml / l
		Cyprinids	0.3-0.8 ml / l
		Cichlids, Characids	± 0.4 ml / l
		Catfishes	0.4-0.8 ml / l
		Eels	0.8-1.0 ml / l
<u>Drawbacks:</u>	<ul style="list-style-type: none"> - Irritations of epithelial tissues - Little margin between induction and toxicity in salmonids 		
<u>Toxicity</u>	<ul style="list-style-type: none"> - Damages the liver and kidney at sublethal doses in mammals, and possibly in fish - Acute toxicity in some species 		
<u>Permanence, legal aspects:</u>	<ul style="list-style-type: none"> - unknown - not approved for fish food (FDA) 		
<u>Suggestions</u>	Prepared syringes for use in natural environments		

<u>Usual name</u>	Quinaldine	<u>Exact name</u>	2-methylquinoline
<u>Synonyms:</u>	none		
<u>Conditioning:</u>	- Transparent liquid, with low solubility in water but high solubility in organic solvents (alcohol, acetone)		
<u>Conservation:</u>	- Opaque bottle and cap (oxidation by air and light)		
	<u>Typical concentrations:</u>	Salmonids	5-12 mg / l
		Cyprinids	2,5-20 mg / l
		Cichlids, Characids	20-40 mg / l
		Catfishes	30-?? mg / l
		Eels	?? mg / l
<u>Drawbacks:</u>	<ul style="list-style-type: none"> - long delay between immersion and injection - fish still sensible to tactile stimuli - no action at pH < 6.0 - irritation of epithelia of operators - strong, persistent odour - strong inter individual variability of responses to anaesthesia 		
<u>Toxicity</u>	<ul style="list-style-type: none"> - increases with water temperature and alkalinity - suspected as carcinogen for operators (larynx, pharynx) 		
<u>Permanence, legal aspects:</u>	<ul style="list-style-type: none"> - no residue in fish muscles after 24 h - accumulation in adipose tissue - not approved for fish food (FDA) 		
<u>Suggestions</u>	<ul style="list-style-type: none"> - solutions (60 % acetone, 40 % water) are highly stable, even in the long run - elimination of tactile reflexes by a preliminary injection of a relaxing compound (gallamine triethiodide, pancurorium bromide,...) 		

<u>Usual name</u>	Quinaldine sulphate	<u>Exact name</u>	Quinate
<u>Synonyms:</u>	No usual synonym		
<u>Conditioning:</u>	- Light yellow crystalline powder, with high solubility in water		
<u>Conservation:</u>	- Opaque bottle and cap (oxidation by air and light)		
<u>Typical concentrations:</u>		Salmonids	25-40 mg / l
		Cyprinids	< 75 mg / l
		Cichlids, Characids	15-60 mg / l
		Catfishes	?? mg / l
		Eels	?? mg / l
<u>Drawbacks:</u>	<ul style="list-style-type: none"> - inconvenience typical of acid solutions (see Tricaine) - fish still sensible to tactile stimuli - irritation of epithelia of operators 		
<u>Toxicity</u>	<ul style="list-style-type: none"> - increases with water temperature and alkalinity - suspected as carcinogen for operators (larynx, pharynx) 		
<u>Permanence, legal aspects:</u>	<ul style="list-style-type: none"> - no residue in fish muscles after 24 h - not approved for fish food (FDA) 		
<u>Suggestions</u>	- buffer the solution prior to use (see tricaine)		

<u>Usual name</u>	Benzocaine	<u>Exact name</u>	Ethyl aminobenzoate

<u>Synonyms:</u>	<i>p</i> -aminobenzoic acid ethyl ester, 4 aminobenzoic acid ethyl ester, ethyl- <i>p</i> -aminobenzoate		
<u>Conditioning:</u>	- Powder with low solubility in water but high solubility in organic solvents (acetone, alcohol)		
<u>Conservation:</u>	- Opaque bottle and cap (oxidation by air and light)		
	<u>Typical concentrations:</u>	Salmonids	25-50 mg / l
		Cyprinids	25-150 mg / l
		Cichlids, Characids	25-100 mg / l
		Catfishes	?? mg / l
		Eels	?? mg / l
<u>Drawbacks:</u>	- High variability of delay between immersion and induction depending on fish size and water temperature - Long recovery, especially in warm water species		
<u>Toxicity</u>	- increases with water temperature increase - No specific toxicity at the concentrations above		
<u>Permanence, legal aspects:</u>	- variability between species, accumulation in muscles - not approved for fish food (FDA)		
<u>Suggestions</u>	- buffer the solution prior to use (see tricaine)		
<u>Usual name</u>	Carbon dioxide	<u>Exact name</u>	Carbon dioxide
<u>Synonyms:</u>	CO ₂ , Carbonic acid, carbonic gas, carbonic anhydride		
	- non combustible gas non combustible, stored at -35°C (solid), or as sodium		

<u>Conditioning:</u>	bicarbonate (NaHCO ₃ , powder); dissolved in water (6.75 %), with addition of sulphuric acid (3,95 %) to obtain the desired concentration in carbonic acid, at a pH in between 7 and 9											
<u>Conservation:</u>	<ul style="list-style-type: none"> - no particularity for bicarbonate - low temperature for CO₂ 											
	<u>Typical concentrations:</u>	<table border="1"> <tr> <td>Salmonids</td> <td>150-650 mg / l</td> </tr> <tr> <td>Cyprinids</td> <td>150-650 mg / l</td> </tr> <tr> <td>Cichlids, Characids</td> <td>?? mg / l</td> </tr> <tr> <td>Catfishes</td> <td>?? mg / l</td> </tr> <tr> <td>Eels</td> <td>?? mg / l</td> </tr> </table>	Salmonids	150-650 mg / l	Cyprinids	150-650 mg / l	Cichlids, Characids	?? mg / l	Catfishes	?? mg / l	Eels	?? mg / l
Salmonids	150-650 mg / l											
Cyprinids	150-650 mg / l											
Cichlids, Characids	?? mg / l											
Catfishes	?? mg / l											
Eels	?? mg / l											
<u>Drawbacks:</u>	<ul style="list-style-type: none"> - mainly used for sedation - risk that the operator loses conscience at _ 10 % CO₂ in the air - risk inherent to the use of sulphuric acid - risk inherent to the use of low temperature for solid CO₂ - hard to obtain deep anaesthesia, and to maintain the oxygen level 											
<u>Toxicity</u>	- risk inherent to hypercapnia in fish, especially with respect to osmoregulation											
<u>Permanence, legal aspects:</u>	<ul style="list-style-type: none"> - No permanence - approved for fish food (FDA) 											
<u>Suggestions</u>	mixing O ₂ and CO ₂ in pressurised cylinders to obtain stable concentrations											